Stepwise Preparation and Characterization of Ultrathin Hydrogels Composed of Thermoresponsive Polymers

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ABSTRACT: Sequential surface chemical reactions of poly(acrylic acid-*co*-*N*-isopropylacrylamide) [poly(AAc-*co*-NIPAAm)] with AAc contents of 5, 10, and 15 mol %, of which carboxyl groups were previously activated by 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC), plus poly(vinylamine hydrochloride) through amide linkages produced ultrathin films on a solid substrate. Assembly processes were quantitatively monitored by a quartz crystal microbalance as substrates. Assembled amounts increased with decreasing both AAc and EDC amounts. Subsequent immersion of ultrathin films into aqueous media resulted in the thickness increases, producing ultrathin hydrogels. Swelling ratios were estimated by percent increases in the thickness and increased with increasing AAc and EDC amounts. Swelling ratios were regularly changed by varying the ionic strength and pH of aqueous media. Swelling properties were interpreted on the basis of structural information on ultrathin hydrogels. Cyclic voltammetries using potassium ferricyanide revealed that ions permeated ultrathin hydrogels, and permeabilities were clearly suppressed above a lower critical solution temperature (LCST) of polyNIPAAm. Reversible on−off changes in permeabilities below and above a LCST were potentially observed. Not only structural control but also stimuli responsive functions of ultrathin hydrogels were realized within the present study.

Introduction

Hydrogels are soft materials that swell as they absorb large amounts of water molecules. These unique materials have applications in both technological and biomedical fields. Water-soluble functional molecules can be reserved and their release regulated via changes in the conditions of outer environments, concomitant with changes in physical properties of hydrogels. From a biomedical or biotechnological point of view,[1] the surfaces of hydrogels proffer the potential to create bioinert (e.g., nonactivated/nonadhesive biomolecules) devices due to their water-swollen structures, which are similar to biointerfaces such as proteins, cells, and tissues. Accordingly, it is potentially valuable to fabricate material surfaces composed of ultrathin hydrogels with a regulated nanostructure. However, it is difficult to create functional coatings from hydrogels using conventional methodologies.

Assemblies composed of structurally regulated polymers on material surfaces enable not only functional modification of the materials but also physicochemical analyses of polymeric characteristics in nanometer-ordered spaces. A layer-by-layer (LbL) assembly has recently been developed to create polyelectrolyte multilayers on surfaces by simple alternate immersion of materials into aqueous solutions of interactive polyelectrolytes including synthetic and biopolymers.[2] Not only ionic interactions but also hydrogen bonds,[3] charge transfer,[4] and van der Waals[5] interactions have been utilized to create functional ultrathin films. LbL assembly is normally applied using noncovalent interactions. However, several research groups have performed chemical reactions to create stable multilayers, in which the component polymers are covalently bound to each other at the interface between film layers.[6]

The swelling of polyelectrolyte multilayers in aqueous ionic solutions has already been investigated in detail.[7] Schlenoff et al.[7c] demonstrated that ionic pairs formed between constituent polyelectrolytes were partially dissolved in aqueous solutions of sodium chloride, resulting in both the increase in film thickness and change in surface topology of films. The swelling was strongly dependent on the salt concentration and the chemical structure of polymers. Rubner et al.[8] demonstrated that multilayers prepared by either electrostatic interactions or hydrogen bonds swelled in buffered physiological solutions depending on both the chemical structure of polymers and assembly conditions (pHs and ionic strength), and the amounts of adherent cells on multilayers were clearly modulated, resulting in expression of cytophilic and cytophobic surfaces. Accordingly, the swelling analysis of multilayers is significant not only for understanding physical properties of multilayers but also for modifying biomedical material surfaces.

On the other hand, the formation of the small number of covalent bonds in multilayers composed of suitable water-soluble polymers permits fabrication of swelled multilayers on surfaces due to the strong hydration of hydrophilic polymer units, which did not join chemical bonds and interpolymer interactions.[9] These multilayers are thought to be ultrathin hydrogels that spread two-dimensionally on surfaces. The creation of these mul-

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tilayers requires the selection of suitable polymer combinations. The sequential amide formation between poly(vinylamine-*co*-*N*-vinylisobutyramide) [poly(VAm-*co*-NVIBA)] with suitable amounts of the VAm units and poly(acrylic acid) (polyAAc) was used to create ultrathin hydrogels.[9a] Furthermore, the obtained hydrogels showed reversible change in hydrophilicity, dependent on the temperature of the aqueous media. This thermoresponse was derived from the phase transition of the polyNVIBA units from coiled to globular states above a lower critical solution temperature (LCST). The sequential reaction was also applied to a combination of poly(vinylamine-*co*-*N*-vinylformamide) and polyAAc for the preparation of ultrathin hydrogels.[9b] Although 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) was used for the activation of carboxyl groups in polyAAc, the maximum amount added to an aqueous polyAAc solution was 5 mol % relative to the total number of carboxyl groups due to polymer precipitation. Furthermore, approximately 40 and 30 mol % of VAm units in poly(VAm-*co*-NVIBA) and poly(VAm-*co*-NVF), which were dependent on steric hindrance of lateral groups, were necessary for sequential chemical reactions. Accordingly, we could not analyze the detailed water-swelling structural properties of ultrathin hydrogels with different amounts of cross-links using the combinations described above. Although the combination of poly(acrylic acid-*co*-acrylamide) and poly(vinylamine) was recently utilized for the preparation of ultrathin hydrogels, some polymers were desorbed at each assembly step, resulting in the zigzag increase in assembly amounts.[9c]

In the present study, combinations of poly(acrylic acid-*co*-*N*-isopropylacrylamide)s [poly(AAc-*co*-NIPAAm)s] with different AAc contents and poly(vinylamine hydrochloride) (polyVAm) were selected for sequential chemical reactions on substrate surfaces to prepare ultrathin hydrogels with different structural properties. Then, effects of EDC amounts, ionic strength, solution pHs, and AAc contents on swelling properties were analyzed to reveal both internal structures and responses to external environments. In addition, thermoresponsive on−off ion permeabilities of ultrathin hydrogels, which derived from a LCST of polyNIPAAm,[11] were also analyzed to reveal stimuli-responsive functions of ultrathin hydrogels.

Experimental Section

Materials. Poly(AAc-*co*-NIPAAm)s with AAc contents of 5, 10, and 15 mol % were synthesized according to methods described in a previous study.[11] Although it was difficult to estimate the molecular weight, AAc content was analyzed by ^{1}H NMR spectra. PolyVAm (M_w 25 000) was purchased from Polysciences. The pH of aqueous polyVAm solution was adjusted from 2 to 7.5 using aqueous 10 mM NaOH solution, dialyzed in water, and then lyophilized. Although vinylamine hydrochloride units are less reactive with activated carboxyl groups than free vinylamine, otherwise much greater amounts of polymers were deposited, resulting in difficulty of swelling analysis. EDC (>98.0%) was purchased from Wako (Japan) and used without further purification. Methyl orange (Wako) and methylene blue (Aldrich) were used without further purification.

Quartz Crystal Microbalance (QCM) Substrate. The assembly was analyzed quantitatively by a 9 MHz QCM, as previously reported in our studies.[5,9] The crystal (9 mm in diameter) was coated on both sides with gold electrodes 4.5 mm in diameter, the roughness of which was 1.7 nm. The frequency was monitored by an Iwatsu frequency counter (model SC7201). The leads of the QCM were protected with a silicone rubber gel in order to prevent degradation during immersion in the aqueous solutions. The amount of polymer deposited, Δm (ng), was estimated by measuring the frequency shift of the QCM, ΔF (Hz), using Sauerbrey's equation[12] as follows:

$$-\Delta F = \frac{2F_0^{\,2}}{A\sqrt{\rho_q \mu_q}}\,\Delta m$$

where F_0 is the parent frequency of the QCM (9×10^6 Hz), A is the electrode area (0.159 cm^2), ρ_q is the density of the quartz (2.65 g cm^{-3}), and μ_q is the shear modulus (2.95×10^{11} dyn cm^{-2}). This equation was reliable when measurements were performed in air as described in this study. Before the reaction, the QCM electrodes were treated three times with a piranha solution [concentrated H_2SO_4/H_2O_2 (30 wt % in water) = 3/1, v/v] for 1 min, followed by rinsing with pure water and drying with N_2 to blast clean the electrode surface.

Sequential Reaction. The cleaned QCM was immersed in an aqueous poly(AAc-*co*-NIPAAm) solution (0.05 unitM) containing EDC (in adequate amounts relative to the total AAc units), in which the carboxyl group had been already activated for 5 min before immersion. The QCM was immersed for 15 min, rinsed gently with pure water, and then dried under N_2 gas. The frequency shift was then measured in air. The QCM was immersed again into an aqueous polyVAm solution (0.05 unitM), and the same procedure was repeated. This sequential reaction cycle was repeated for the preparation of ultrathin hydrogels. The pHs of aqueous solutions of poly(AAc-*co*-NIPAAm) with AAc contents of 5, 10, and 15 mol %, and polyVAm were 4.3, 4.0, 4.0, and 7.5, respectively. Assembly was performed at 4 °C to maintain the activity of EDC. Although assembly was started with poly(AAc-*co*-NIPAAm), it was possible to initiate assembly with polyVAm. Assembly was stable even though polymers at the first step were physically adsorbed without any chemical reaction onto the QCM substrate.

Characterization. AFM images were obtained with a Digital Instruments NanoScope III that was operated in contact mode in both air and water at ambient temperature. We did not perform any image processing other than flat leveling. The swelling ratio was obtained by the following equation:

$$\mathrm{SW} = \frac{d_{\mathrm{water}}}{d_{\mathrm{air}}} \times 100$$

where SW is swelling ratio (%), d_{water} is the film thickness in water, and d_{air} is the film thickness in air. Attenuated total reflection (ATR) spectra were obtained with a Perkin-Elmer Spectrum One in air at ambient temperature. One side of a poly(ethylene terephthalate) film was coated with gold to obtain a reflective surface. Polymers were then assembled using a method similar to that used in the QCM measurement. The interferograms were coadded four times and Fourier transformed at a resolution of 4 cm^{-1}. Dye adsorption experiments were basically followed by a previous study.[13] Ultrathin hydrogels were similarly prepared on a transparent quartz substrate (45 × 9 × 1 mm). The coated substrate was immersed into dye solutions at 1 mM (respective pHs of methyl orange and methylene blue were 7.6 and 4.8) for 15 min at 20 °C, rinsed with pure water, and then dried with nitrogen gas. Dye amounts adsorbed were analyzed by an ultraviolet−visible (UV−vis) spectrometer, Jasco model V-550, at ambient temperature. Cyclic voltammetries (CVs) were measured by ALS/CH-Instruments electrochemical analyzer model 660 using potassium ferricyanide under a nitrogen atmosphere. Potassium ferricyanide was dissolved into 0.1 M sodium perchlorate at a concentration of 10 mM. Double-sided QCM electrodes were used as a working electrode. A polished Pt wire 1 mm in diameter and Ag/AgCl (saturated aqueous NaCl solution) were used as counter and reference electrodes, respectively, as cited in our previous study.[9b]

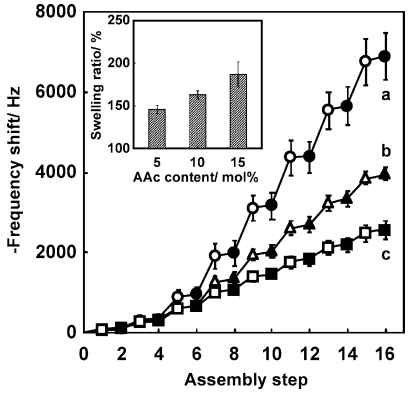

Figure 1. QCM analysis of the stepwise assembly of poly(AAc-*co*-NIPAAm) with AAc contents of (a) 5, (b) 10, and (c) 15 mol %, plus polyVAm in the presence of 1.0 equimolar amount of EDC to AAc units at 4 °C. The open and closed symbols show the poly(AAc-*co*-NIPAAm) and polyVAm steps, respectively. The inset shows swelling ratios of 16-step assembled ultrathin hydrogels, which were estimated by the following equation: [(thickness in water)/(thickness in air) × 100].

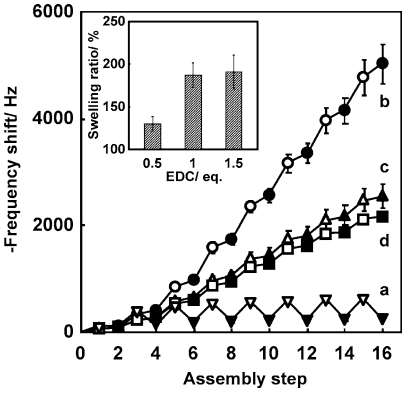

Figure 2. QCM analysis of the stepwise assembly between poly(AAc-*co*-NIPAAm) with an AAc contents of 15 mol % and polyVAm in the presence of (a) 0, (b) 0.5, (c) 1.0, and (d) 1.5 equimolar amounts of EDC. The open and closed symbols show the poly(AAc-*co*-NIPAAm) and polyVAm steps, respectively. The inset shows swelling ratios of 16-step assembled ultrathin hydrogels, similarly estimated.

Results and Discussion

Covalently constructed polyelectrolyte multilayers partially composed of polymers containing neutral and hydrophilic units potentially transformed into ultrathin hydrogels in aqueous media due to the swelling of water molecules into multilayers.[9] For various applications of ultrathin hydrogels, it is important to control physicochemical properties (e.g., amounts of functional groups, swelling ratio, and permeation of molecules) on the basis of chemical strategies. A reasonable method is adequate selection of polymer components. We have already applied polymers of *N*-vinylalkylamides,[9a,b] of which the side groups could be partially hydrolyzed to introduce primary amines, and copolymers of acrylamide and AAc[9c] to a single component of multilayer formation. The preparation of ultrathin hydrogels was potentially performed using those polymers from the point of a difference in chemical structures. Since preparative conditions were limited, and it was difficult to control physicochemical properties using those polymers, we selected the present combination of poly(AAc-*co*-NIPAAm)s and polyVAm and characterized assembly processes, swelling properties, incorporation of charged dyes, and thermoresponsive ion permeabilities.

Figure 1 shows frequency shifts corresponding to assembly amounts plotted against assembly steps, when poly(AAc-*co*-NIPAAm)s with AAc contents of 5, 10, and 15 mol % were sequentially assembled with polyVAm on a QCM substrate in the presence of an equimolar EDC amount to the AAc units. The mean roughness analyzed by AFM was 2.0 ± 0.5, 2.5 ± 0.5, and 3.7 ± 0.3 nm for 16-step assemblies of copolymers with AAc contents of 5, 10, and 15 mol %, respectively, resulting in the preparation of smooth ultrathin films. Assembly processes were reproducible in all cases, and frequencies decreased with increasing numbers of steps, indicating stepwise deposition of polymers. ATR spectra of assemblies demonstrated disappearance of a weak peak for carbonyl vibration bands at around 1710 cm^{-1}, corresponding to carboxyl groups in AAc units, which had been observed for a cast film of copolymers. This observation suggests the formation of amide linkages between poly(AAc-*co*-NIPAAm)s and polyVAm during assembly processes. Furthermore, the films obtained were stable and did not desorb in aqueous media, and the alternate immersion of the substrate in the absence of EDC did not demonstrate the stepwise change in frequency (see Figure 2a), also supporting the formation of chemical bonds between polymers. In the present system, the swelling of water molecules basically derives from the presence of neutral NIPAAm units of the copolymers. In other words, since multilayers electrostatically constructed from polyAAc and polyVAm did not swell with water molecules in pure water, it is better to reduce ion complexes in assemblies for simple construction of ultrathin hydrogels. From this point of view, acrylamide copolymers containing small amounts of AAc units might be suitable for multilayer components compared to copolymers of *N*-vinylalkylamides.[9a,b]

Total amounts assembled were dependent on AAc units and increased with decreasing AAc units. Although amounts of assembled polyVAm analyzed by the QCM method were almost the same in all cases, amounts of copolymers increased with decreasing AAc units. Accordingly, the difference was derived from increased assembly amounts of copolymers. This is possibly due to conformational differences of copolymers in reaction solution. A decrease in AAc content relaxes electrostatic repulsion of units, forming a more coiled conformation in aqueous media. When coiled polymers reacted with polyVAm on the assembly surface, the apparent monolayer thickness increases, assuming deposition with a similar two-dimensional density. In other words, extended polymers with a greater AAc content resulted in the construction of thinner monolayers on the assembly surface. These conformational effects have already been similarly demonstrated in cases of electrostatic LbL assembly.[13a,14] Furthermore, total assembled numbers of AAc units, which were estimated from total assembly amounts of copolymers, percent contents of AAc units, and mean unit molecular weights of copolymers, were independent of AAc content and were the same within experimental error for all copolymers. This estimation indicates that the increase in assembly amounts of copolymers is derived from an increase in coiled NIPAAm segments, which had not participated in film-based reactions, in the films. On the other hand, total assembled numbers of VAm units similarly estimated were approximately 1.5 times greater than those of AAc units in all cases, indicating an excess

presence of amino groups within films. In fact, UV−vis spectral analyses demonstrated that an anionic dye, methyl orange, was clearly incorporated into films, while a cationic dye, methylene blue, was not incorporated at all under the same conditions. This observation also supports that cationic VAm units remain in films and suggests that almost all AAc units react with VAm units during assembly. Note that the incorporation of methyl orange was independent of the outermost polymer component.

Apparent swelling ratios were estimated as percent increases in thickness in air and water at ambient temperature, as shown in the inset of Figure 1. The thickness in water increased compared to that in air in all cases, indicating swelling with water molecules. The dependence of swelling ratios against AAc content was characteristic for ultrathin hydrogels prepared by LbL assembly. The ratio increased with increasing AAc content. Since swelling of conventional bulk hydrogels is normally suppressed by increased cross-links, swelling of ultrathin hydrogels may decrease with increasing AAc content. Accordingly, swelling properties cannot be simply interpreted by the number of cross-links. Conventional hydrogels are prepared from homogeneous solutions of monomers or polymers, while ultrathin hydrogels are prepared by stepwise two-dimensional deposition of polymer monolayers. In this case, not only cross-links that formed in films but also the conformation of deposited polymers possibly affected swelling properties. As already shown above, VAm, NIPAAm, and newly formed amide groups exist in ultrathin hydrogels. Considering that those units are hydrophilic, the conformational effect seemed to be a major factor for the difference in swelling ratios. Copolymers with greater AAc contents, which had been assembled under more extended conformations, had the ability to swell in water because copolymers can be changed into coiled conformations after swelling. In other words, since the copolymers with smaller AAc contents were already comprised of coiled conformations in air, the thickness increase seemed to be suppressed. On the basis of these conformational effects, the difference in the apparent swelling ratios, depending on AAc contents, can be interpreted.

Changes in EDC amounts for stepwise reactions further represent assembly manners. Figure 2 shows frequency shifts corresponding to assembly amounts plotted against assembly steps, when poly(AAc-*co*-NIPAAm)s with 15 mol % AAc content was sequentially assembled with polyVAm on the QCM substrate at various EDC amounts. Above an equimolar EDC to AAc units, assembly amounts were almost the same, indicating that similar assemblies had occurred. On the other hand, the amount was clearly increased at 0.5 equimolar EDC. Since the same copolymer was used here, the difference in polymer conformations in solutions cannot reasonably explain the increased amount. These data suggest conformational changes of copolymers on film surfaces depend on EDC amounts. The reaction of all AAc units with VAm units on the film should necessitate changes of the copolymer to more extended conformations. However, when EDC is insufficient, conformational changes are incomplete. Accordingly, greater amounts were assembled at 0.5 equimolar EDC. These analyses suggest another regulation of physicochemical properties of ultrathin hydrogels. In fact, the swelling ratio of the film prepared at 0.5 equimolar EDC was clearly smaller than others, as shown in the inset of Figure 2. This is possibly due to the presence of partially formed ionic complexes of remained AAc and VAm units (the film becomes hydrophobic due to complexes, similar to a manner of polyion complexes). The presence of AAc units within the film was also supported by slight incorporation of methylene blue into the film (data not shown). Furthermore, the swelling ratio was smaller than that of the film prepared from the copolymer with 5 mol % AAc content at an equimolar EDC, even though apparent cross-links for both films are similar. This also supports the presence of hydrophobic ionic complexes. It is noted that swelling ratios of films prepared at above an equimolar EDC are the same, also indicating fabrication of the same film. As a consequence, an equimolar EDC was sufficient to prepare ordinary ultrathin hydrogels, and decreasing amounts of EDC resulted in varied swelling properties.

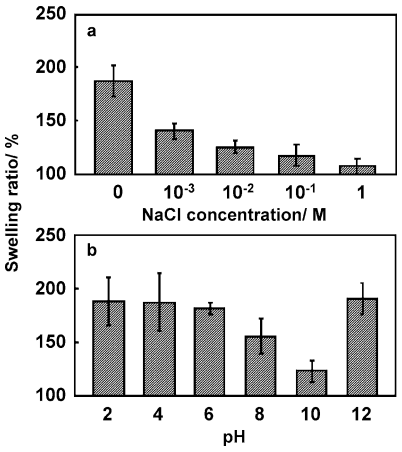

Figure 3. Swelling ratios of the 16-step assembled ultrathin hydrogel prepared from poly(AAc-*co*-NIPAAm) with an AAc content of 15 mol % and polyVAm in the presence of 1.0 equimolar amount of EDC in aqueous media at various (a) NaCl concentrations and (b) pHs. The pH was adjusted by using aqueous 10 mM HCl and NaOH solutions, and then the ionic strength was maintained.

Swelling properties of polyelectrolyte multilayers determine their potential functions. Rubner et al. demonstrated that swelling of multilayers, of which the swelling ratios could be controlled by assembly conditions using a single polymer combination, can regulate cytophilic/cytophobic properties.[8] Accordingly, it is significant to analyze the swelling of ultrathin hydrogels under various atmospheres. Figure 3a shows swelling ratios of the ultrathin hydrogel prepared from poly(AAc-*co*-NIPAAm) with an AAc content of 15 mol % and polyVAm in the presence of an equimolar EDC amount to AAc units, when it was immersed into various NaCl concentrations at ambient temperature. Swelling ratios gradually decreased with increasing NaCl concentration. This is possibly due to deswelling of water molecules from polymer components by coexistent inorganic ions. Furthermore, Figure 3b shows swelling ratios of the same film when it was immersed into aqueous solutions at various pHs. The swelling was clearly suppressed at pH 10. As it has already discussed, VAm units remain in the film. In addition, the pK_a of VAm units is known to be 10.0.[15] Accordingly, deprotonation of VAm units occurr above this pH, and VAm units become more hydrophobic, thus resulting in a decrease in swelling. It is difficult to reasonably explain increased swelling at pH 12. In our previous study,[16] dehydro-

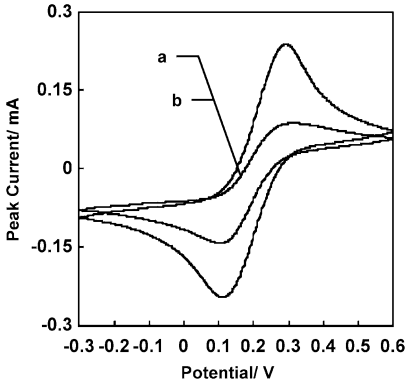

Figure 4. CV charts of (a) a bare QCM and (b) the eight-step assembled ultrathin hydrogel prepared from poly(AAc-*co*-NIPAAm) with an AAc content of 15 mol % and polyVAm in the presence of 1.0 equimolar amount of EDC at a sweep rate of 0.05 V s^{-1}.

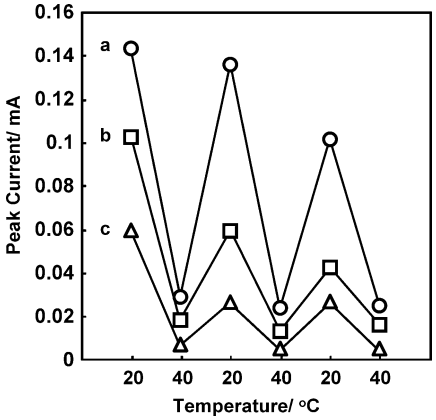

Figure 5. Reversible changes of CV peak currents at 20 and 40 °C at a sweep rate of 0.05 V s^{-1} for the ultrathin hydrogel prepared from poly(AAc-*co*-NIPAAm) with an AAc content of 15 mol % and polyVAm in the presence of 1.0 equimolar amount of EDC: (a) 8-, (b) 12-, and (c) 16-step assembly.

genation reactions using polyVAm-protected platinum nanoparticles suggested further deprotonation of amino groups, resulting in anionically charged amines. Regeneration of charges seemed to be the reason for the increase. From these observations, the swelling of ultrathin hydrogels could be regulated by ionic strength and pH of aqueous media, depending on the chemical structure of constituent polymers.

Permeabilities of small molecules are sensitive to the structure of ultrathin hydrogels. Since dyes could be incorporated into ultrathin hydrogels as shown above, these small molecules should be permeable. Furthermore, temperature-dependent permeabilities are expected due to thermoresponsible properties of polyNIPAAm segments. Here, we analyzed permeabilities of potassium ferricyanide using a CV technique, which analyzes permeabilities of electroactive ionic species across films deposited onto an appropriate electrode on the basis of oxidation−reduction cycles. QCM plates coated with ultrathin hydrogels were directly used for the electrode. Before analyses of on−off permeability, CVs of ultrathin hydrogels with different structures are obtained at 20 °C, of which the temperature is lower than a LCST of polyNIPAAm, approximately 32 °C.[17] Typical CVs are shown in Figure 4. A suitable CV curve that contains oxidation and reduction processes was obtained for a bare QCM electrode, and the peak current was 0.242 mA cm^{-2}. A similar curve containing the peak current of 0.132 mA cm^{-2} was observed for the electrode coated with ultrathin hydrogels. Latter peak currents were smaller than former ones, even though anionic ferricyanide ions may be electrostatically condensed to the present amine-rich ultrathin hydrogels. In fact, our previous study demonstrated the peak current for carboxyl group-rich ultrathin hydrogels was similar to a bare electrode.[9b] These observations suggest that initially adsorbed copolymers more strongly interact with the electrode surface, and then the electrode area seems to become apparently small. On the other hand, peak currents gradually decreased with increasing thickness of ultrathin hydrogels, indicating that ionic diffusion was suppressed with increasing thickness. It is also noted that the peak current of the ultrathin hydrogel prepared at 0.5 equimolar EDC was greater than those prepared at 1.0 and 1.5 equimolar EDC, of which the assembly amounts were adjusted to be almost the same by changing assembly steps. This observation indicates that ionic species can more readily permeate into ultrathin hydrogels with smaller amounts of covalent cross-links (see above). Furthermore, the dependence of peak currents against the square root of the sweep rate demonstrated linear relationships in all cases, thus indicating that CVs were governed by diffusion of electroactive ions into ultrathin hydrogels. As a consequence, potassium ferricyanide potentially permeated ultrathin hydrogels through water in films to react with the electrode.

For analyzing on−off ionic permeabilities, CVs were measured at various temperatures between 20 and 60 °C, at which temperature ultrathin hydrogels stably remained on the substrate. Peak currents steeply decreased between 30 and 35 °C because of the deswelling of polyNIPAAm segments and subsequent shrinkage of polymer chains on the substrate, resulting in the suppression of ionic permeabilities. Furthermore, reversible changes of peak currents at 20 and 40 °C were observed as shown in Figure 5. Permeabilities were significantly controlled by an on−off manner in all cases, and the films were stably remained after cycles. The on−off magnitude was greater for thinner hydrogels because the peak current below a LCST was greater. Peak currents gradually decreased with increasing cycles. This is possibly due to gradual changes in internal nanostructures of ultrathin hydrogels, followed by the difficulty of complete regeneration of initial structures. Preliminary experiments of contact angles using air bubbles in water demonstrated that the surface became hydrophobic above the LCST, also indicating deswelling of water molecules. These observations suggest that ultrathin hydrogels can be used for various stimuli-responsive nanomaterials by modification with functional molecules in/on hydrogels.

Conclusions

Ultrathin hydrogels were prepared by stepwise monolayer deposition of poly(AAc-*co*-NIPAAm)s with different AAc contents and polyVAm on solid substrates through chemical reactions using EDC to activate AAc units and were characterized by suitable methods. QCM substrates were used to quantitatively monitor assembly processes. Sequential reactions under various conditions demonstrated potential regulation of hydrogel structures. Smaller AAc contents led to greater assembly amounts; however, swelling ratios increased with increasing contents. Less than an equimolar amount of

EDC to AAc contents led to greater assembly amounts; then swelling ratios decreased. Swelling ratios were affected by ionic strength and pH of aqueous media. These physicochemical properties of ultrathin hydrogels were interpreted in terms of differences in chemical structures of constituent polymers, polymer conformations in aqueous media and on surfaces, polymer hydrophobicity, and reaction efficiencies between polymers. Electroactive ionic species permeated ultrathin hydrogels, and the permeation was suppressed above a LCST of polyNIPAAm. Furthermore, on−off permeation was reversibly observed. Preliminary experiments demonstrated facile incorporation of various organic small molecules and metal nanoparticles into ultrathin hydrogels, and these properties will be reported in the near future. Ultrathin hydrogels allow for novel surface modification for various technological applications.

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